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THE SEXUAL BEHAVIOR OF ANURA

6. THE MATING PATTERN OF *BUFO AMERICANUS*, *BUFO FOWLERI*, AND *BUFO TERRESTRIS*^{1, 2}

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INTRODUCTION

The mating activities of many species of the genus *Bufo* are relatively better known than are those of most other species of Anura. This is due to three major reasons: (1) the relative abundance of many *Bufo* species, (2) the ease with which they may be studied in the field or captured during and after the breeding season, and (3) the slow rate at which their sexual activity declines when kept in captivity. There are, nevertheless, a number of confusing and contradictory statements in the literature dealing with the sexual activity of toads which call for elucidation. The purpose of this study was to investigate the

sexual behavior of *Bufo americanus* and related species, particularly those phases which lent themselves to laboratory research.

Bufo americanus was found to be most suitable for this study, and this species was used for the longer experiments and more extensive observations. Shorter comparative observations were made on the other two species, *Bufo fowleri* and *Bufo terrestris*.

Most of the experiments were conducted during the 1940 breeding season under the guidance of the late Dr. G. Kingsley Noble.

MATERIALS AND METHODS

The American toads were collected in various localities in New York State and New England during April and May. The Fowler's toads all came from Long Island, mostly from Long Beach, and a few from the vicinity of Middle Village. The southern toads were purchased from a dealer in Englewood, Florida, during May. They were kept in large wire cages containing earth, sand, gravel, damp moss, and pans of water, and were fed daily with blow flies (*Calliphora*) and meal worms (*Tenebrio*). Fowler's toads were heavily parasitized, and a large number of them died. *B. americanus* and *terrestris* were

much more viable, but the females of the latter species were least active sexually.

The female American toads ovulated spontaneously. Some Fowler's toads ovulated after homoplastic pituitary injections (Rugh, 1935). Only one partial ovulation of *Bufo terrestris* was obtained after pituitary treatment. It is probable that most of the latter species were collected shortly after breeding and the ovaries had not developed sufficiently when the pituitary injections were started. That pituitary implantations are ineffective in *Rana pipiens* immediately after the breeding season has been noted by Rugh (1937).

Most of the tests (except where noted) were conducted in glass-walled slate or mirror-bottomed aquaria, 30 cm. × 40 cm. × 25 cm. filled to a depth of approximately 4 cm. with water at room temperature. A few stones, twigs, and pieces of

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straw in the water completed the set-up. At first the observations were conducted in a dark room with a dim red light for illumination, but it was soon discovered that the toads reacted equally as well in

broad daylight. Thereafter no attempt was made to darken the testing room.

Further details on methods will be given in the description of the experiments which follow.

VOICE

Three distinct calls were recognized in the males of the species studied, namely, the sex trill, the chirp, and the warning vibration. As contrasted with *Rana pipiens* (Noble and Aronson, 1942), these three vocalizations were so distinct that it was almost impossible to confuse them. There were, in addition, slight variations in the homologous calls in the three species studied, so that after some experience it was possible to identify the species on the basis of any one of the three calls.

The fright cry heard in many species of *Rana* (Boulenger, 1897; Dickerson, 1906; Noble and Farris, 1929; Noble and Aronson, 1942) was never heard during this study. It has been described by Wright and Wright (1942) as occurring rarely in *B. terrestris*.

The females of the species studied are mute. This is apparently a general characteristic of the genus, as no reports were found of any female *Bufo* ever vocalizing. When the observer held a female by its back, markedly increased respiratory (thumping) motions were often noted. In the male, these respiratory changes were associated with the chirp and warning vibrations, but in the female no sound resulted. Savage (1934) noted a similar response in *Bufo bufo*. Many gravid females did not exhibit these respiratory exaggerations.

MALE SEX TRILL

The sex call or trill of the male is the well-known voice which is heard in the ponds during the toad breeding season. It is the primary component of the toad chorus. This call has been described adequately in the literature (Dickerson, 1906; Wright, 1914, 1932; Wright and Wright, 1942; Blair, 1942), and no further description is required here. The sex call is seasonal. It is sounded most frequently at the height

of the breeding period; as the season progresses it is sounded less and less frequently, and after the season it is seldom heard. Environmental factors, particularly rain and changes in temperature, are believed to stimulate the sex call of the male during the breeding season (Bragg, 1941).

The function of the sex call in *Bufo* has been described as attracting the female as well as other males (Courtis, 1907; Miller, 1909; Wellman, 1917; Noble, 1931; Savage, 1934; Locher, 1939; and Bragg, 1940, 1941). It has been pointed out by Bragg (1942) that in some species of Anura, e.g., *Bufo cognatus*, there is apparently a delayed response of the females to the calls of the male which may amount to several hours or days. Experiments by Blair (1942) indicated that given the choice of two choruses of related species (*B. woodhousii* and *B. americanus*), males and females were attracted to a limited extent by the call of their own species.

Various species of *Rana* will call while floating, spread out on the surface of the water (Bragg and Smith, 1942), or while swimming towards a female (Noble and Aronson, 1942). In contrast to this, the toads under consideration were never observed to call while floating or swimming, and always stopped calling before clasping a female.

MALE CHIRP

The chirp is the familiar sound which is elicited when the back of the male is touched. It is approximately the pitch of the sex trill, but of much shorter duration, and is repeated continually until the "annoyance" ceases. If the "annoyance" lasts for a long time, the chirping sometimes ceases for short intervals. Chirping is often accompanied by a partial inflation of the vocal sac. The function of the chirp

is not known. It is not effective in causing a clasping male to release the chirper (see section on sex recognition and discussion).

MALE WARNING VIBRATION

This call is readily elicited whenever the back of a male is stimulated. It is a dull, vibratory, barely audible sound, which can be heard only if the toad is held within a few inches of one's ear. On the other hand, if one touches the back of a vibrating toad the vibrations can easily be felt. This vibration functions independently of the vocal cords, since we found that severance of the latter did not eliminate the vibration. The physiological mechanism involved is not known. It is suggested that the accentuated respiratory movements cause the entire arytenoid cartilage to vibrate, and these vibrations are transmitted through the body musculature. This warning vibration is very effective

in causing a second clasping male to release immediately (see section on sex recognition).

Both the chirp and the warning vibration are seasonal and were less readily elicited after the breeding season, the warning vibration being the more persistent of the two. Listed in order of disappearance after the season, the sex trill is first, the chirp second, and the warning vibration last. Sickness, inanition, and other disturbing factors also caused the vocalizations to disappear in the above-named order.

It should be noted that the chirp and warning vibration were elicited by the same stimuli, but the chirp was generally the less easily evoked of the two. Stimuli adequate to elicit the chirp always brought forth the warning vibration, but the converse did not hold, particularly towards the end of the season.

RESPONSE OF A MALE TO A CLASP OBJECT

In the experiments on *Rana pipiens* previously reported (Noble and Aronson, 1942) it was demonstrated that within the limits of an experimental situation sexually active males did not exhibit any discrimination of males, females, or pairs at a distance as shown by attempts at clasping. Since investigators claiming such discriminatory reactions utilized species of the genus *Bufo* for their observations, it was deemed advisable to repeat this experiment. Hinsche (1926) claimed that male *Bufo vulgaris* responded to certain motions peculiar to the female. Savage (1934) credited the male *Bufo bufo* with the ability of recognizing a pair. "... The lower member is certainly a female ... A male which attacks a pair has some chance of dislodging his rival. Both the capacity for recognizing a pair and the advantage of attacking it seem to exist."

For the experiment herein reported a large aquarium was used, 110 cm. \times 50 cm. \times 31 cm., filled with water to a depth of 8 cm. at 23° C. The room was darkened and the tank was illuminated from above by a single 25-watt bulb. The following Amer-

ican toads were introduced: three pairs, three gravid females, and three sexually inactive males. A sexually excited male (experimental male) was now selected (one forcibly separated from a female) and introduced into the tank. The number of times the experimental male attempted to clasp males, females, or pairs was recorded. Attempts at clasping females were often successful, in which case the newly formed pair was separated. When the attempt was made to clasp a pair, the observer reported whether the action was directed towards the male or female of the pair. When the responses of the experimental male showed a reduced frequency, he was replaced by another active individual. Each trial was conducted for 20 minutes.

The results of the experiment are summarized in table 1. It should be noted that this is a record of attempts at amplexus, and no distinction was made between successful and unsuccessful endeavors. If the experimental males chose at random between the three clasp objects, the percentage of times that they clasped each clasp object would be 33.3, and for 409 trials the standard error, $\epsilon(p)$ of this *a priori*

TABLE 1

DISTANCE DISCRIMINATION OF PAIRS, MALES, AND FEMALES, BY SEXUALLY EXCITED MALES AS SHOWN BY ATTEMPTS AT CLASPING

Trial		Total number of attempted clasps	Number of times male tried to clasp				
			Pairs			Males	Females
			Male of pair	Female of pair	Total for pairs		
1	54	21	7	28	13	13	
2	43	13	2	15	12	16	
3	44	19	1	20	20	4	
4	35	13	0	13	8	14	
5	37	18	0	18	13	6	
6	24	3	1	4	8	12	
7	22	7	1	8	10	4	
8	30	21	0	21	4	5	
9	61	32	0	32	10	19	
10	33	10	0	10	14	9	
11	26	7	1	8	10	8	
N = 409		M% = 96.8	M% = 3.2	Σ = 177 = 43.3%	Σ = 122 = 29.8%	Σ = 110 = 26.9%	

$\epsilon(p)$ = standard error of a 33.3% *a priori* random probability.

$$\epsilon(p) = \pm \sqrt{\frac{33.3 \times 66.7}{409}} = \pm 2.3\%.$$

random probability would be ± 2.3 per cent. The actual percentages of times that the males clasped males or females fell within two and one-half times the standard error, and hence no significant deviation from the expected ratio was indicated. The percentage of times that pairs were clasped was greater than $3\epsilon(p)$, indicating a slight but significant preference for the pairs. Thus the observation of Savage (1934) that there is a greater tendency for males to select pairs is substantiated. On the other hand, this experiment contradicts Savage's conclusion as to the relative advantage of attempting amplexus with a pair. It will be noted that of the 177 attempts to clasp pairs, 96.8 per cent were directed to the male of the pair, and all ended in failure. Of the 3.2 per cent of the attempts to clasp the female of the pair, only two clasps (0.5 per cent) were

successful. Of these one was on the ventral side of the female. In the second case the new suitor still had the task of displacing the original possessor, and the intruding male showed no particular advantage in the lengthy struggle that ensued.

As Courtis (1907) and Hinsche (1926) had previously noted, the motions (activity) of the pairs, males, and females appeared to be the most important factor in attracting the male providing, of course, that the clasp objects were near enough to be visible. In this experiment the pairs were generally most active, the males next, while the females often rested quietly on the bottom.

While this experiment was not repeated on *Bufo fowleri* and *terrestris*, observations indicated that a very similar mechanism existed in these species.

SEX RECOGNITION

The literature contains suggestions that the sex discriminatory mechanism is not an efficient one, that males are frequently found clasping other males, or the males of pairs (Liu, 1931; Rugh, 1935). To ex-

amine this question, the results of the attempts at amplexus of the previous experiment were tabulated. When the clasp was successful, two minutes were allowed to elapse before the pair was forcibly

TABLE 2

RESULTS OF ATTEMPTS AT AMPLEXUS WITH MALES, FEMALES, AND WITH BOTH MEMBERS OF A PAIR

Clasp object	Total number of attempts	Per cent of attempted amplexus showing		
		Immediate release*	Slow release**	Forcibly separated***
Male of pair	- 164	100.0	0.0	0.0
Female of pair	13	84.6	0.0	15.4
Separate male	110	100.0	0.0	0.0
Separate female	122	25.4	5.7	68.9

* Within 10 seconds. ** Between 10 seconds and 2 minutes. *** After 2 minutes.

separated. Release was considered immediate if it occurred within ten seconds. Releases occurring between ten seconds and two minutes were recorded as slow releases. The results of this experiment are indicated in table 2. Of the 164 attempts of the experimental males to clasp males of pairs, and of the 110 attempts of the experimental males to clasp separate males, all (100 per cent) resulted in the immediate release of the clasp object. In contrast to this, 68.9 per cent of the 122 attempts of the experimental males to clasp females were successful, and the newly formed pairs had to be forcibly separated. Of the 13 attempts to clasp the females of pairs, only two trials (15.4 per cent) were successful. This experiment demonstrates clearly the efficiency of the discriminatory mechanism which functions after amplexus has been attempted.

To investigate further the mechanisms involved in post-amplexus sex recognition (the discrimination of a male from a female after amplexus is attempted), a series of experiments were performed using the 30 cm. \times 40 cm. \times 25 cm. test tanks. In these experiments the clasp object was modified in various ways, so as to eliminate one by one the possible factors which might cause a male attempting amplexus to release. In this manner it was possible to "highlight" the effective mechanism, and at the same time to eliminate, or at least to reduce in importance, certain factors which heretofore were considered important in sex recognition. Each of these experiments was repeated at least ten times on *Bufo americanus* with similar results, and was repeated several times on

the other two species. The sexually active experimental males used in the following experiments were first allowed to clasp females for at least five minutes. They were forcibly separated from the females just before being tested with various clasp objects.

Exp. 1: A male was injected intraperitoneally with 5 cc. of physiological saline solution, thus increasing his girth to approximately that of a fat female. This treatment did not affect the chirp or the warning vibration of the injected male. A sexually active experimental male (just separated from a female as noted above) was placed in a tank with the saline-injected male. The experimental male attempted amplexus several times with the saline-injected male, but released immediately after each attempt. From this experiment it is concluded that increasing the girth of a male in order to approximate that of a gravid female does not enable a sexually active male to maintain his clasp.

Exp. 2: A spent female was now introduced into a test tank. This female had recently laid a large complement of eggs and was thinner than the average male. A sexually active experimental male was placed in the tank with the spent female. Amplexus soon occurred and lasted for 20 minutes when the male was forcibly separated. Hence, reducing the girth of a female is ineffective in causing a clasping male to release.

Exp. 3: A male was anaesthetized by immersion in a 3 per cent solution of urethane. This treated male did not move and could not be stimulated to sound

the chirp or warning vibration. A sexually active experimental male was now placed in the tank with the anaesthetized male, and the latter was pushed around by the observer until the experimental male clasped. Amplexus was maintained for about 20 minutes. At this time the urethane began to wear off, and the treated male began to sound the chirp and warning vibration.

As a supplement to experiment 3, a dead male was placed in a tank with an experimental male, and the body was pushed around by the observer until the sexually active male clasped. Amplexus was maintained until the two were forcibly separated after a half hour. From these experiments it is concluded that some reaction not present in a dead or anaesthetized male stimulates a clasping male to release. Moreover, the effective physiological mechanism is probably the chirp or warning croak (or both).

Exp. 4: A male was muted by cutting the vocal cords. This operation eliminated the chirp but not the warning vibration. An experimental male was introduced into a tank with the muted male. The sexually active male attempted amplexus several times but released immediately each time. Of the two physiological factors suggested by the previous experiment, it is now evident that the warning vibration alone may be an effective stimulus for release.

Exp. 5: A male was partially anaesthetized by immersing it in a urethane solution for a short time. This treatment eliminated the chirp but not the warning vibration. When the experimental male was introduced he attempted amplexus several times but released immediately each time. Again it is concluded that the warning vibration alone may be the effective factor causing a clasping male to release.

Exp. 6 (Bufo americanus only): A male was wrapped in several layers of crepe paper. Following this procedure, the male chirped almost continuously, but the warning vibration could not be felt through the wrappings by the observer. A sexually active male was now placed on the wrapped

male, and despite the chirping and the awkward shape of the clasp object, amplexus lasted over 15 minutes. It is now concluded that the chirp is not effective in causing a clasping male to release and that the warning vibration alone is the effective factor.

Exp. 7: An experimental male was induced to clasp the female of a pair by wedging himself between the original male and the female. The pectoral regions of both males were in contact with the back of the female. The hind limbs of the original male rested on the head and back of the newcomer, thereby stimulating the second male to chirp and give a more or less continuous warning vibration. The observer then touched the back of the original male so that now both males chirped and gave the warning vibration. Both males remained clasping until forcibly separated after a half hour. This experiment indicates that the effective warning vibration is not an auditory stimulus but a tactual one, and the effective region of the clasping male for the reception of the stimuli is apparently the ventral pectoral area and the inner surfaces of the forelimbs.

Exp. 8: The observer firmly held the hind limbs of a male and placed the animal on the surface of the water in an observation tank. An experimental male was introduced and attempted amplexus many times with the restrained male. Immediate release followed each attempt. The observer then tried placing the experimental male on the restrained male, but the former would not clasp. Liu (1931), Savage (1934), and Locher (1939) have postulated a "repulsive action" which is a characteristic of the male. According to these authors a male will struggle and try to dislodge a second clasping male, while the females are passive and will permit a clasping male to retain his clasp. The present results indicate that the "repulsive action" is not a major factor in sex recognition, since a restrained male was quickly released.

The results of the above series of experiments are summarized as follows:

(1) Differences in girth among a gravid

female, a spent female, or a male are not factors in post-amplexus sex recognition.

(2) The chirp does not function in this capacity.

(3) The warning vibration of the male is the only apparent mechanism by which a male distinguishes a female from another male after amplexus is attempted.

(4) Although the warning vibration can be heard and felt by the observer, the clasping male responds primarily to tactual stimulation (not to auditory stimulation), and the region of the clasping toad sensitive to these stimuli is the ventral pectoral region plus the medial surfaces of the forelimbs.

AMPLEXUS

As already noted by Wright (1914, 1932), amplexus was either axillary or supra-axillary. According to our observations the type of clasp seemed to depend on the relative sizes of the male and female. In figure 1 it is clearly supra-axillary.

The hind limbs of the male were fully flexed, and the male's abdomen adhered closely to the back of the female, his back arched convexly. The tip of the male's mouth generally reached the transverse level of the eyes of the female. For ex-

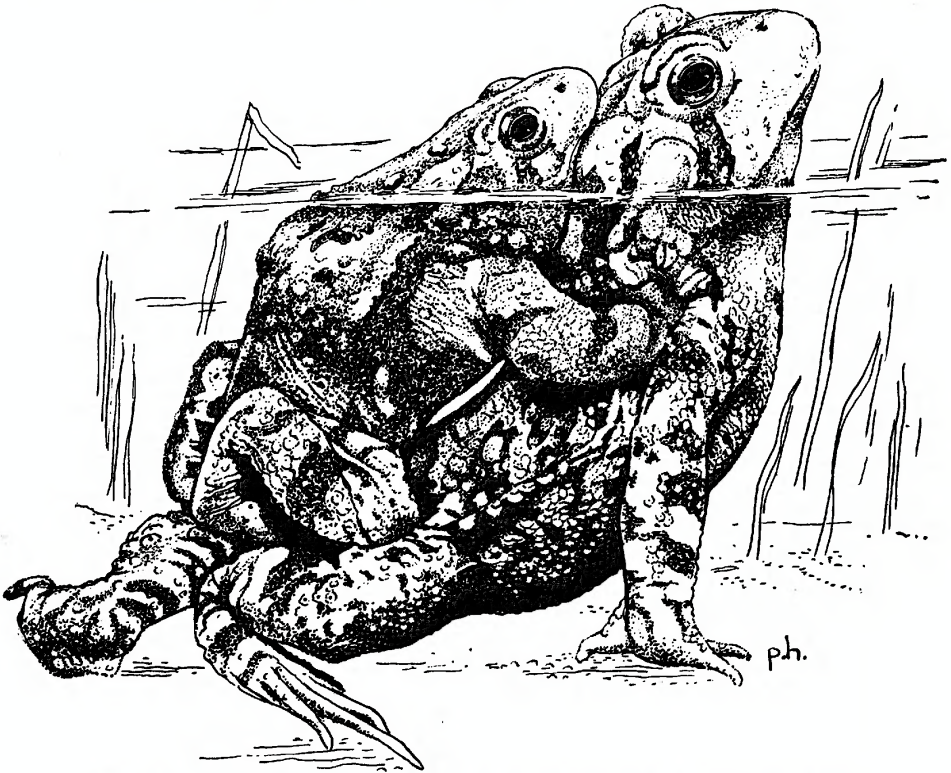


Fig. 1. *Bufo americanus* pair exhibiting the back-arch release mechanism.

Some of the *Bufo terrestris* females used were more than twice the size of the males. In these cases the clasps were all supra-axillary. Where the male and female were of the same size, axillary clasps usually resulted.

cellent illustrations of various clasping *Bufo*, the reader is referred to Roesel von Rosenhof (1758, pl. 20), Boulenger (1897), Wright (1914), Hinsche (1926), Wright (1932), Savage (1934), Rugh (1935), and Bragg (1937).

OVIPOSITION

Descriptions of the egg-laying process in various species of the genus *Bufo* are numerous and date far back in biological literature. Roesel von Rosenhof (1758) described in considerable detail and with an excellent colored plate the oviposition of a toad which he called *Bufo terrestris, dorso tuberculis exasporato, oculis rubris* (probably *Bufo bufo bufo*). He also wrote a brief description of the oviposition of *Bufo terrestris foetidus* (*Bufo calamita*). Similar descriptions of the spawnings of these two species were presented by Spallanzani (1785) and Héron-Royer (1885, 1886). Boulenger (1897) described the oviposition of *Bufo vulgaris* (probably *Bufo bufo bufo*); Miller (1909) and Wright (1914) reported on the oviposition in *Bufo americanus*; Wright (1932) witnessed the spawning of *Bufo terrestris*; Liu (1932) reported on *Bufo raddei*, while Rostand (1934) and Savage (1934) described anew the egg laying of *Bufo bufo bufo* including certain new interpretations.

These descriptions reveal that the mode of oviposition is remarkably similar in these species of *Bufo* as contrasted with the divergent mechanisms of spawning in *Rana* and *Hyla*.

The following description of the oviposition of *Bufo americanus*, though somewhat repetitious, is presented in an attempt to clarify some of the discrepancies in the literature. It is based upon a series of spawnings of *Bufo fowleri* and *Bufo americanus* and one partial spawning of *Bufo terrestris*. No differences were noted in the egg-laying behavior of the three species.

Pre-oviposition activity (during amplexus but prior to the actual spawning) consisted of (1) a slightly increased restlessness on the part of the female, and (2) peristaltic-like contractions of the female's abdominal muscles. These very characteristic movements generally occurred as a series of from three to twelve waves over a period of a few minutes. In most cases they started posteriorly, simultaneously on both sides, and travelled anteriorly. Occasionally they occurred alternately, first on one side and then on the

other. On a few occasions they were noted to start anteriorly and to travel caudally. Following these contractions, the female generally moved to a new location.

The pre-oviposition period varied from a few minutes to many hours. It was followed by the oviposition or in some cases by a period of pseudo-oviposition (see p. 10) before the actual egg deposition started. The female always started the egg laying by arching her back concavely and by extending her hind limbs caudally (fig. 2), thighs pointed outward, legs inward, heels close together, or touching at the midline, and toes pointed outward. Thus a diamond-shaped enclosure was formed by the female's hind limbs. This position resembled to a limited extent the oviposition posture of female *Rana pipiens* (Noble and Aronson, 1942) and *Rana septentrionalis* (Aronson, 1943).

Immediately following this movement of the female, the male arched his back convexly and partially extended his hind limbs, knees pointed downward and slightly forward, resting on the outer surface of the female's thighs. The legs pointed medially and dorsally; the ankles pointed upward, the heels downward, while the toes pointed ventrally and extended under the female's cloaca. The exact position of the male's knees and toes varied somewhat, apparently depending on the size relationship of the male and female. The resemblance of the male's position to the oviposition posture of a female *Rana pipiens* is very striking. The result of this posturing of the male and female was that their cloacas were brought into juxtaposition.

The female's abdominal wall now contracted (as a whole and not like the peristaltic movements described above), and a clump of eggs issued from her cloaca. Almost simultaneously the male's abdominal walls showed three or four brief ejaculatory contractions. The egg mass often remained for a few minutes just behind the male and female's cloacas, supported by the toes of the male. Then, sliding over the latter, they fell within



Fig. 2. Oviposition of *Bufo americanus*.

the diamond-shaped enclosure formed by the female's hind limbs.

The female then gradually spread her heels apart and soon assumed her normal crouching position followed by a similar action of the male. The female now moved

about from place to place for two to ten minutes, spreading out the eggs and thus revealing their arrangement in two strings.

One end of each of the strings was free, while the other ends extended into the female's cloaca. A series of peristaltic

contractions, as described above, now followed, and a new oviposition cycle was started. These cycles sometimes lasted less than a minute and usually occurred every three to ten minutes. During some of these spawning cycles the male and female remained in the oviposition posture for a number of minutes after the extrusion of the eggs. In such cases the peristaltic waves often occurred while the pair were in this posture, and it sometimes happened that a second egg extrusion started before the pair moved from the egg-laying posture.

The duration of two complete spawnings was recorded. One (*Bufo americanus*) lasted three hours and thirty minutes. The other (*Bufo fowleri*) took five hours and ten minutes. Some of the spawnings of all three species were of a multiple type. That is, the egg string broke (ovulation apparently ceased), and the male released the female. Then, after a variable length of time, the female was reclasped by the same or a different male, and the egg laying was resumed. In one case, two such interruptions were noted.

RELEASE

The release of the female by the male at the termination of the spawning was observed in *Bufo americanus* on three occasions. The release, which will be described below, is representative of the other two occasions where this behavior was witnessed.

A female who had laid a small part of her eggs during the night was placed in an observation tank with a sexually active male at 10 A.M. Amplexus occurred almost immediately. At 10:25 A.M. the first oviposition cycle occurred, and similar cycles recurred at intervals of three to ten minutes until 11:35 when the last typical cycle was noted. At 11:40 another oviposition cycle started, but very few eggs were laid, and the egg chains broke. At 11:45 another oviposition cycle took place, which was typical in every respect, including the male's ejaculatory contractions, except that no eggs were emitted. At 11:47 another cycle started. No eggs were emitted; but this time the female arched her back severely (fig. 1), much more than in the typical spawning cycle, and the male was partially lifted off the back of the female. The male immediately released his clasp of the female. Within one

minute the male reclasped the female. Thirty seconds later an oviposition cycle was started, no eggs appeared, the female arched her back severely, and the male released. This reclasping and release was repeated a second time within the next two minutes.

In the second case the male started calling immediately after releasing the female, and this vocalization continued until he reclasped the female. Three post-spawning reclasps and releases were witnessed with this pair.

The reaction of several recently spent females (spawned within the preceding 48 hours) to clasping males was studied. The males readily maintained tight clasps upon these females. After intervals of two to fifteen minutes, oviposition cycles occurred, the females arched their backs severely (no eggs were laid), and the males released after from one to five cycles. Thus far this back-arch release mechanism has been noticed only in recently spent females. When males clasped unovulated females or females that spawned one or two months previously, no release behavior was elicited even after many hours or days in amplexus.

PSEUDO-OVIPOSITION

Bufo fowleri and *Bufo terrestris* females were injected with two to four homoplastic anterior pituitary bodies, in an attempt to induce ovulation. In many cases ovulation did not occur, but when these females

were placed in tanks with active males, the females were clasped, and pseudo-oviposition often took place. Pseudo-oviposition differed from normal oviposition in the following respects: (1).

peristaltic contractions of the female's abdominal walls were seldom evident; (2) no eggs were extruded; (3) the pseudo-oviposition cycles generally occurred at

far less frequent intervals (15 minutes to one hour) than the normal oviposition cycles, and (4) the back-arch release mechanism did not appear.

DISCUSSION

SEX RECOGNITION

Although reports of males maintaining clasps on other males have been made frequently (Liu, 1931; Rugh, 1935), our results indicate that this is not a general procedure of male toads in captivity. Moreover, field observations usually refer to only a few such pairs (Wright, 1914), whereas there are often hundreds or thousands of male toads located in a small breeding area. Nevertheless, some explanation of these "mistakes" is necessary. It was pointed out that the warning vibration of the male, which is the all-important mechanism in sex recognition, becomes weaker or disappears completely as a result of sickness, inanition, or the passing of the breeding season. It is probable that such males are the ones that are clasped. Examination in the laboratory of pairs consisting of two males invariably showed that the clasped male was not sounding the warning vibration, and after the pair was separated the warning vibration was never elicited when the investigator touched the back of the formerly clasped male.

When toads are confined to laboratory habitats, weaknesses, and sickness are exaggerated. Toads so affected will rapidly lose their warning vibration, and because of crowded conditions it is not long before they are clasped by the healthier toads. It is not surprising, therefore, to find some males being clasped by other males. We feel that this is the explanation for the statement by Rugh (1935) that sexually aroused male toads show a lack of discrimination by maintaining amplexus with other male toads, whereas male frogs show no such lack of discriminatory powers. If one wishes to compare the sexual discriminatory ability of our local *Bufo* and *Rana*, it must be concluded that the toads are at least as well equipped for the task as are the frogs.

The literature on the mechanism of sex

recognition in *Rana* has been reviewed recently by Noble and Aronson (1942). In this genus the warning croak and girth of the toad being clasped are the major factors involved. This is probably true for most species of the genus, but according to Savage (1934) a totally different discriminatory mechanism is found in *Rana temporaria*.

Investigators working with various species of *Bufo* (Miller, 1909; Liu, 1931; Savage, 1934; and Löcher, 1939) with the exception of Hinsche (1926) are in agreement that the relative girths of the male and female are not factors in sex discrimination. The present study confirms this conclusion. The above authors (Hinsche again excepted) are also in agreement that the warning voice or chirp is a very important factor. In none of these studies has the distinction been made between the chirp and the warning vibration. From the descriptions of the voice and the allusions to auditory functions, it is presumed that these workers were referring to the chirp. We have found, to the contrary, that the chirp is not an effective mechanism in causing a clasping male to release; this function is carried out by the barely audible warning vibration, and it is clearly not an auditory but a tactile response. Owing to the close relation of the warning vibration and chirp, it is clear how confusion might have arisen. Our conclusions are limited to the three species under investigation.

Yerkes (1905), while studying the sense of hearing in frogs, could not find any motor reactions resulting directly from auditory stimulation. By experimentation he found that sounds will modify reactions resulting from visual or tactual stimuli. The release of a vibrating male is clearly the result of tactual stimuli. It is possible that the sound of the chirp reinforces this motor action of release. This

might conceivably be revealed by more refined measurements of the release time.

A "repulsive action, a kind of dodging dive which males alone display when seized" (Savage, 1934) has also been postulated as a secondary discriminatory factor by Liu (1931) and Locher (1939). We have noticed that both males and females tended to struggle when clasped, but we have been unable to observe any sexual differences in this behavior which could be effective in the sex discriminatory mechanism. Considering the immediate effectiveness of the warning vibrations in cases where the clasped male was restrained by the observer (exp. 8), any "repulsive actions" which might normally be present would have to be relegated to a very minor role.

Hinsche (1926) recognized the importance of tactual stimulation for the maintenance of amplexus, but his interpretation of its function is just the opposite of that given here. This author believed that the roughness of the skin, the pulsations of the flanks, and peculiar locomotion are factors characteristic of a female and which will induce a clasping male to retain his clasp. However, these differential structures and habits are not clearly demonstrated, and it is hard to conceive of any slight differences between the sexes which might exist as the major factors in this relatively efficient mechanism of sex recognition. Hinsche's investigation was orientated mainly towards the problem of why males will sometimes maintain lengthily (many days) clasps on inanimate objects, frogs, salamanders, etc. Here such factors as girth, shape, plasticity, and skin texture undoubtedly play much more important parts.

OVIPOSITION

It is apparent that some of the differences in the descriptions of the egg layings which are found in the literature are due to species variations. Thus the females of the three species considered in this report all formed diamond-shaped enclosures with their hind limbs, while in the European species the females apparently extend fully their lower extremities.

The omissions of the peristaltic contractions in the descriptions of some of the more recent authors (Boulenger, 1897; Miller, 1909; Wright, 1914, 1932; and Savage, 1934) were probably due to incomplete observation or reporting, since other authors observing the same species have found this behavior to be very characteristic. Indeed, the earlier authors Roesel von Rosenhof and Spallanzani described these abdominal contractions in very vivid terms, and the latter author ventured the opinion that these contractions were concerned with ovulation and with the passage of the eggs down the oviduct.

The observations made by Roesel von Rosenhof (1758), Miller, (1909), Wright, (1914, 1932) and verified in the present report, that when the male's feet are hooked behind the female's cloaca they form a basket for the retention of the eggs, are vigorously denied by Savage (1934). This author points out that when the pair was supported by weeds away from the substratum (as generally occurs in the ponds) such retention of the eggs would not occur. However, during the present observations, straw and weeds were plentiful and the pair was often supported by them; yet the "baskets" were often in evidence. The presence or absence of these "baskets" was apparently due to size differences of the male and female, since the position of the male's toes during oviposition and hence the ability of the toes to support the eggs would depend upon this size ratio.

The interpretations of Héron-Royer (1886) and Boulenger (1897), that the male helps to draw the eggs out of the female, have already been refuted by Miller (1909), Rostand (1934), and Savage (1934), and the present study supports this denial.

Savage (1934), in discussing the oviposition of *Rana temporaria*, stated that the stimulus for the male ejaculation is the contact of the outcoming eggs with the pubic region of the male. Likewise for the common toad the contact of the outcoming eggs with the male's toes was considered by Savage as the adequate stimulus for the ejaculation by the male. Noble and Aronson (1942) have shown that

this explanation was not satisfactory for *Rana pipiens*. In the present study ejaculatory movements were observed during pseudo-oviposition, when no eggs were present to act as a stimulus. The work with *Rana pipiens* suggested that the egg-laying movements of the female stimulated the ventral pectoral region of the male to cause the ejaculation. The present study indicates that a similar mechanism is present in the *Bufo* under consideration.

RELEASE AFTER OVIPOSITION

Savage (1934) pointed out that the mechanism whereby the male releases the female at the termination of the oviposition might very well differ in those *Anura* that lay their eggs in a relatively short period of time as compared to those frogs and toads whose oviposition lasts an hour or more. We have also found this to be the case, although our interpretations of the release mechanisms differ from those of Savage. A review of the various explanations of the release after oviposition in *Anura* follows.

Noble and Farris (1929), working with *Rana sylvatica*, hypothesized that the reduction in girth of the female was the responsible factor. Savage (1934), studying *Rana temporaria*, added the reappearance of the warning croak to the hypothesis of Noble and Farris. However, our observations on a number of American *Rana* indicated that the warning croak of the female generally does not return for

several hours after the spawning. Noble and Aronson (1942), observing *Rana pipiens*, suggested the stimulus for release was probably the resultant of a number of factors including (1) the ejaculations of the male, (2) reduction in girth of the female, (3) cessation of the female oviposition reflexes, and (4) movement of the female from the egg-laying posture.

Returning to the toads, Liu (1931) noted that in *Bufo raddei* (as well as in *Rana nigromaculata*) there was a peculiar stretching movement of the female's body which caused the male to release. Savage (1934) claimed that the break in the egg strings—that is, the break in a chain of reflexes which require at a certain point the stimulus of eggs touching the male's toes—causes the male to release.

Hyla andersonii deposit their eggs during an extended series of oviposition cycles. It was noted by Aronson (1944) that when the eggs ceased to flow, still another oviposition cycle occurred. During this cycle, the female arched her back severely, the male was partially raised from the back of the female and released two seconds later. We have found the same back-arch release mechanism in the *Bufo* under consideration. That the presence or absence of eggs is not the important factor was indicated by our extended pseudo-ovipositions, some of which lasted a whole day without the male releasing. It seems likely that the peculiar stretching of the female noted by Liu is a modified form of this release mechanism.

SUMMARY

1. Three distinct vocalizations are recognized in the male toads, namely, the sex trill, the chirp, and the warning vibration. These calls are seasonal, being most easily elicited at the height of the breeding season. After the season they disappear in the following order: sex trill first, chirp second, and warning vibration last.

2. In an experimental situation, sexually active male toads do not discriminate at a distance between other males or females but attempt to clasp members of either sex with equal frequency. There is,

however, a slight tendency to clasp pairs more frequently than individuals, the clasps in these cases being directed almost entirely to the male of the pair. This preference for pairs is probably due to greater activity on the part of the pairs.

3. The sex-recognition mechanism (discrimination of a male from a female after amplexus is attempted) is found to be very efficient. The mechanism depends almost exclusively on the warning vibration of a male being clasped. These vibrations act as tactual stimuli of the ventral

pectoral region and medial surfaces of the forelimbs of the clasping male, causing him to reject the clasp object. Females do not emit these vibrations, and therefore the clasping male maintains amplexus. Relative girths of male and female, relative "repulsive actions" of both sexes, and the male's chirp are found not to be efficacious in sex recognition.

4. The positions and movements of the male and female during the oviposition are described in detail.

5. Release of the female by the male at the termination of the oviposition follows a severe concave arching of the back of the female, which partially lifts the male off the female.

6. Pseudo-oviposition (oviposition movements without the deposition of eggs) can sometimes be induced in anterior pituitary injected but unovulated females when clasped by males.

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